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ABSTRACT

Time Course of Immune Activity in Response to Two Acute Stressors

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A number of studies have examined the effects of acute laboratory stressors on measures of immune system activity, reporting variable and sometimes contradictory results. We collected immune measures at four times during and following two common laboratory stressors (mental arithmetic with harassment and cold pressor) to temporally examine immune response patterns to these stressors. The six-minute stressor period was associated with increased self-report of pain and distress in both groups, as well as increased SBP, DBP and HR in the mental arithmetic group. Increased NK activity in this group was observed at 2 and 5 minutes into the task and at 5 minutes after the task. The cold pressor group exhibited a marginal increase in NK activity 2 minutes into the task. A significant group x time effect was observed on lymphocyte proliferation to three concentrations of pokeweed mitogen.

TIME COURSE OF IMMUNE ACTIVITY IN RESPONSE TO TWO ACUTE STRESSORS

by

Douglas L. Delahanty

Thesis submitted to the Faculty of the Department of Medical and Clinical Psychology Graduate Program of the Uniformed Services University of the Health Sciences in partial fulfillment of the requirements for the degree of Master of Science 1994

Time Course of Immune Activity in Response to Two Acute Stressors

Numerous studies have been conducted examining the effects of acute stressors on immune system activity. Although current reviews (Kiecolt-Glaser, Cacioppo, Malarkey & Glaser, 1992) and meta analyses (Herbert & Cohen, 1993) describe overall patterns of immune response to laboratory stressors, individual analysis of the articles indicates varied and sometimes contradictory results with respect to natural killer (NK) cell activity. Some of the variability in these results is undoubtedly due to the use of different stressors of differing durations and to taking immune measures at varying times during and after exposure to the stressor. The present study examined changes in NK activity during and after two common acute stressors (mental arithmetic with harassment and cold pressor) in addition to the effects of these stressors on lymphocyte proliferation to mitogen challenge.

Many have suggested that stress can have deleterious effects on health and that the immune system may be an important mediator in the stress-health relationship (for reviews see O'Leary, 1990; Jemmot & Locke, 1984; Dorian & Garfinkel, 1987). Animal studies have demonstrated stress-increased changes in resistance to the development and spread of tumors concurrent with stress-decreased immune system activity (Riley, 1981; Sklar & Anisman, 1981; Ben-Eliyahu, Yirmiya, Liebeskind, Taylor & Gale, 1991), and human studies have identified stress as a factor in vulnerability to infection (Cohen, Tyrrell & Smith, 1991). In addition, the strength of the immune system and of immunosurveillance

is thought to mediate the effects of stress on cancer progression in humans (Herberman & Ortaldo, 1981; Steinhauer, Doyle, Reed & Kadish, 1982; Shimokawara et al., 1982). Evidence of stress-induced changes in immune activity is extensive (Kiecolt-Glaser et al., 1992; Herbert & Cohen, 1993), and psychological stress may be a risk factor for the development of colds or infectious mononucleosis (Cohen et al., 1991; Kasl, Evans & Niederman, 1979). However, clear indications of the clinical implications of observed immune changes have not been reported and the dynamics and characteristics of acute stress-related immune changes have not been investigated.

Acute Stressors and NK Cell Activity

While most evidence of stress-related reduction of immune system efficacy comes from naturalistic studies, several recent investigations have examined the effects of acute laboratory stressors on various indices of immune system activity (for review see Kiecolt-Glaser, Cacioppo, Malarkey & Glaser, 1992). However, results of these studies have been extremely variable with the most variable results involving NK cell activity. Although studies consistently report an increase in NK cell number following an acute stressor (Brosschot et al., 1992; Bachen et al., 1992; Naliboff et al., 1991; Landmann et al., 1984), measures of NK cell activity have yielded inconsistent findings.

Sieber et al. (1992) exposed males to repeated controllable or uncontrollable noise stressor sessions. Subjects were exposed to two 20-minute stressor sessions, and subjects in the uncontrollable stressor condition exhibited

decreased NK cell activity immediately after the first session. NK activity remained lower immediately after and 24 and 72 hours following the second exposure. No change in immune activity was observed in the controllable stressor groups.

Cohen et al. (under review) also observed decreased NK activity in men. In this case, subjects were exposed to a mild 30-minute Stroop stressor, and immune measures were taken at 10 and 20 minutes into the task, immediately following the task, and 40 minutes after the task. They found that, at 40 minutes after the task, subjects in the stress condition showed significantly lower NK activity than a group of control subjects. No differences in NK activity were apparent at the other timepoints.

However, Naliboff et al. (1992) observed significant increases in NK activity in young women (21-41 yrs) immediately following a 12 minute mental arithmetic stressor. Subjects were divided into two age groups: 21-41 and 65-85, and each subject attended two experimental sessions. In one of the sessions subjects were exposed to 12 minutes of mental arithmetic and in the other they viewed 12 minutes of a videotaped lecture on a health topic. The younger women had significantly higher NK activity during the mental arithmetic task than during the film. These differences were not evident among older women. Similarly, Knapp et al. (1992) exposed 10 men and 10 women to 40 minutes of either negative or positive emotion elicitation. Subjects in the negative emotion group evidenced a weak, nonsignificant trend indicating increased NK cell activity immediately

following the elicitation session.

NK activity was also examined in first-time tandem parachutists (Schedlowski et al., 1993). Immune measures were taken two hours prior to the jump, immediately after, and one hour after the jump. They found increased NK activity immediately after the jump, however, one hour after the jump NK cell activity was significantly lower than baseline values.

Though apparently contradictory when analyzed individually, these data considered together suggest a biphasic NK response pattern similar to what one would expect if NK activity was affected by opponent processes (Solomon & Corbit, 1974). That is, initial responses appear to be reversed by counteracting pressures once the stressor has ended. Studies that measured NK activity immediately after acute stressors generally reported increases in NK activity, while studies that examined more distal time points have shown stress-related decreases in NK activity. If the effects of stress on NK activity are biphasic, that is, an initial increase followed by a return to or below resting levels, studies that measure NK activity during or immediately after a stressor are likely to produce results that seem contradictory to studies measuring NK activity at more distal time points. The present study provided a detailed account of immediate effects of stress on NK activity.

Acute stressors and lymphocyte proliferation

Despite use of a variety of stressors of differing durations in studies of acute stress, findings consistently show stress-induced decreases in proliferation to

concanavalin A (Con A) and phytohemagglutinin (PHA: Knapp et al., 1992; Weisse et al., 1990; Zakowski, McAllister, Deal & Baum, 1992; Zakowski et al., in press; Zakowski, 1993). Only two studies have examined lymphocyte response to pokeweed mitogen (PWM; Brosschot et al., 1992; Zakowski et al., in press). Brosschot et al. found no change in lymphocyte proliferation after a 30-minute frustrating interpersonal stressor, but Zakowski et al. found decreased proliferation to PWM 30 minutes after subjects viewed 30 minutes of stress-inducing, gruesome films.

Interestingly, Zakowski et al. found different effects of two types of laboratory stressors on mitogen response. Using an active psychological/ performance stressor (20 min of mental arithmetic and the Stroop) they observed a decrease in response to Con A immediately following the stressor that persisted for 30 minutes after the stressor with no effects on PWM. Exposing subjects to a passive film stressor, they observed decreased proliferation to PWM 30 minutes after stressor termination with no change in proliferation to Con A. Active stressors are thought to involve more active participation in the task and appear to evoke higher SBP changes than passive stressors which are believed to require more passive acceptance and sensory intake of the stimulus. Potential mechanisms for the differential physiological effects of these two types of stressors may involve preferential activity of beta versus alpha-adrenergic receptors (Schniederman & McCabe, 1989).

The present study was designed to examine the effects of active and passive

laboratory stressors on NK cell activity and to replicate findings reflecting different immunoproliferative effects of these stressors. It was predicted that NK cell activity would follow a time-dependent biphasic pattern similar to that created by opponent processes (Solomon & Corbit, 1974): an initial increase in activity during and continuing through the duration of the stressor was expected to slowly return to baseline and subsequently decrease to below baseline values. Based on Zakowski et al's (in press) findings, it was predicted that the MA group would show an immediate and sustained decrease in proliferation to Con A and that the CP group would show a delayed decrease in proliferation to PWM.

Methods

Subjects

Subjects were 31 healthy males between the ages of 20 and 45 (mean = 29.5) recruited through an advertisement in a local newspaper; three were African-American, one Asian-American and 27 Caucasians. All subjects were screened by telephone and were excluded if they did not meet inclusion criteria, including nonsmoker status, no reports of drug use and clear history of psychiatric disease, diabetes and hypertension. Subjects were called the night before the study to ensure that they had not recently experienced symptoms of illness.

Design

Subjects were randomly assigned to one of three stressor groups: MA (N=11), CP (N=10) or control (N=10). Control subjects read magazines during the stress period. The study was a 3 (group: MA, CP, control) x 6 (time: baseline,

during instructions, 2 min and 5 min into a 6 min stressor, and 5 min and 15 min post stressor) mixed design. Major dependent variables included NK activity and lymphocyte proliferation to Con A and PWM at these times. Cardiovascular and self-report measures were collected to determine effectiveness of the stress manipulation, and several questionnaires were administered to assess subjects' backgrounds and ambient stress levels.

Procedure

Experimental sessions were held weekdays beginning at 7am. Upon subjects' arrival at the laboratory, the study was explained and informed consent was obtained. A heparinized catheter was then inserted into the antecubital vein of the nondominant arm and a self-inflating cuff and blood pressure/heart rate monitor (Accutracker, Suntech) was attached to the dominant arm to measure these functions. After initial setup, subjects rested for 30 minutes, read magazines and completed background questionnaires. Heart rate and BP measures were taken every five minutes during the rest period, and 20 minutes into the rest period a baseline blood draw was taken.

Following the rest period, instructions for the tasks were given. To keep the instructions for the experimental tasks constant, they were recorded, and the tape was played for each subject. A separate experimenter who had no other contact with the subject conducted the task, operating the tape recorder and addressing questions. The control group was simply told that their task was to read magazines for the six minute task period. Subjects in the MA group were

told, "Shortly you will be performing a mental arithmetic task. This is the kind of task that is typically used for aptitude tests. I will tell you a four digit number, and your task is to start counting backwards by 7's as quickly and accurately as you can. For example, I may say the number 1176 and you would say 1169, 1162, 1155, and so on as fast as you can until you are told to stop. At this point I will tell you another four digit number and you will begin subtracting from that number. Remember, it is very important that you work as quickly and accurately as possible, or we will not be able to use the data we collect." At this time the experimenter stopped the tape, asked for questions, and reiterated the importance of working quickly and accurately. The tape was started again, and the subject was told, "The task will begin now. Begin counting backwards by 7's from the number 1276." The sound of a metronome ticking was used to enhance the stressor, and the experimenter continually harassed and prodded the subjects to go faster and be more accurate.

The tape for the CP group instructed the subjects as follows: "The task you will be completing is a cold pressor test. That is, for the next 6 minutes I will periodically ask you to immerse your dominant hand into a basin of cold water for variable amounts of time. You are to put your hand all the way into the water without touching the bottom of the basin until I tell you to take it out. If you do take your hand out earlier, we will have to stop the experiment because we will not be able to use your data. Do you have any questions?" The water for the task was kept at 3°C, and between the variable submersion times (duration of

immersions = 30, 20, 45, 15, 40, 30 seconds), the subjects removed their hands for 30 seconds.

All task sessions lasted for six minutes, and blood draws were taken during the instruction period, two minutes into the task, and five minutes into the task. In addition, heart rate and blood pressure measures were taken every two minutes during the task. Following termination of the stressor subjects answered questions that served as manipulation checks including measures of mood and perceived task stressfulness. In addition, subjects completed a modified version of the Schedule of Recent Events (Holmes & Rahe, 1967). Two more blood draws were taken (5 and 15 min post-termination of the stressor), and heart rate and blood pressure were recorded every five minutes after the stressor. After the final blood draw subjects were paid $30 and debriefed.

Measures

Background questionnaires were designed to determine basic history and demographic information as well as recent health and diet. The Beck Depression Inventory (BDI; reliability =.86; Beck, Ward, Mendelson, Mock & Erbaugh, 1961) was administered to determine if subjects were experiencing symptoms of depression since depression has been shown to have an immunosuppressing effect (Irwin, Daniels, Smith, Bloom & Weiner, 1987). The Symptom Checklist 90-R (SCL-90R; Derogatis, 1977) was used to determine symptom distress and to allow us to control for differential subject experience. Subjects indicated which of a list of 90 symptoms they had experienced within the preceding week and how severely

the symptoms were rated. The SCL-90R is composed of nine subscales including somatization, obsessive-compulsive, interpersonal sensitivity, depression, anxiety, hostility, phobic anxiety, paranoid ideation and psychoticism (test-retest reliabilities ranging from .80 for anxiety to .90 for phobic anxiety), and was used to control for symptoms or distress that may be affecting immune system activity.

Current mood and mood change following the stressor was measured with the Profile of Mood States (POMS; McNair, Lorr & Proppleman, 1971) which includes the dimensions of tension-anxiety, depression-dejection, anger-hostility, vigor, fatigue, and confusion-bewilderment. Reliabilities of the subscales range from .84 to .95 (Eichman, 1978). In addition, immediately following the task subjects were asked to rank how distressing and how painful the task was on six-point Likert-type scales (0=no stress or pain - 5= extreme stress or pain). Together with BP and HR, these measures were used to determine the efficacy of the stressors.

Immune Measures

Procedures for immune assays were adapted from Kiecolt-Glaser et al. (1984). Samples were collected into heparinized glass tubes, and 20 ml of blood from each sample were layered onto 10 ml of Ficoll-Hypaque (Pharmacia Fine Chemicals, Piscataway, NJ). Samples were then centrifuged at 1800 rpm for 30 minutes. Lymphocytes were harvested from the interface and were washed twice. Cells were washed and incubated in RPMI medium with Hepes (Mediatech, Herndon, VA) supplemented with 10% heat inactivated fetal calf serum (Gibco,

Grand Island, NY), 1% glutamine (200 mM, Gibco), 1% penicillin-streptomycin (Gibco), 1% Beta-mercaptoethanol (2 x 10^{-3}M, Sigma), 2% Hepes (1M, Sigma) and 3% sodium bicarbonate (7%, Sigma). Cells were counted using Trypan Blue exclusion criteria, and concentration was adjusted to 8 x 10^6 cells/ml. The cells were then centrifuged and resuspended in 0.8 ml of supplemented RPMI to give a final cell density of 1 x 10^7 cells/ml.

Target cells were prepared from ongoing K562 cell cultures. The cells were pulsed with 150 mCi of Na_2CrO_4 (^{51}Cr: New England Nuclear Research Products, Boston, MA.) 16 hours prior to the start of the assay. Target cells were washed twice and counted, and concentrations were standardized to 1 x 10^5 cells/ml at the start of the assay.

Lysis of target cells was determined in a 5hr chromium release assay. Effector cells were set up in a v-bottom 96 well plate (Costar, Cambridge, MA) by serial dilution to achieve effector/target (E:T) ratios of 50:1, 25:1, 12.5:1 and 6.25:1. Each sample was run in triplicate. Additional wells contained target cells with media only and target cells with 5% sodium dodecyl sulfate (SDS, Sigma) to determine spontaneous and maximum release of radioactivity, respectively. Plates were then centrifuged at 1000 rpm for 5 min, and incubated for 5 hours with 5% CO_2 at 37°C. The supernate was harvested using an SCS Harvesting Set (Skatron Instruments, VA). Level of ^{51}Cr released in each sample was reported in decays per minute (DPM) using a Beckman 9000 gamma counter. Percent of total possible lysis for each E:T ratio was calculated by dividing the sample DPM minus

the spontaneous release by the maximum DPM minus the spontaneous release.

Lymphocytes for the proliferation assay were prepared in the same manner although the cells were washed and incubated in RPMI 1640 medium with Hepes (Mediatech, Herndon, VA) supplemented with 2 mM glutamine, 100 U/ml streptomycin, 1mM sodium pyruvate, 0.1 mM non-essential amino acids, 5×10^{-5} M 2-mercaptoethanol, and 5% heat inactivated fetal calf serum. Cells were counted using Trypan Blue exclusion criteria, and concentration was adjusted to 2×10^{6} cells/ml. Con A (Sigma Medical Company, St. Louis, MO) was used at concentrations of 5ug/ml, 7.5ug/ml and 10ug/ml, and PWM (Sigma) was used at concentrations of 0.01 ug/ml, 0.05 ug/ml and 0.1 ug/ml. Background proliferation was measured by incubating cells in complete media only, and each assay was performed in triplicate. Con A or PWM (100 ul) was added to 100ul of complete media containing 2×10^{5} lymphocytes in 96 well, flat-bottomed plates (Costar, Cambridge, MA). Plates were then incubated for 52 hours with 5% CO_2 at 37°C. After incubation, the cells were pulsed with 20ul of tritiated thymidine per well (50uCi/ml; ICN Pharmaceuticals, Inc., Irvine, CA) and incubated an additional 18 hours. The cell cultures were then harvested onto glass fiber filter paper using a PHD cell harvester (Cambridge Technology, Inc., Watertown, MA). A Beckman LS5801 liquid scintillation counter was used to assess the incorporation of [^{3}H]-thymidine into newly synthesized DNA in stimulated and nonstimulated cultures. Values were expressed as disintegrations per minute (dpm) and were averaged across each triplicate. Additionally, values from the nonstimulated cultures were

subtracted from those of the mitogen stimulated samples in order to determine responsiveness to Con A and PWM alone.

Results

Initial ANOVAs were conducted to determine if baseline differences in background and demographic variables existed between groups. To evaluate the effectiveness of the stressors ANOVAs were conducted on the manipulation checks and mood ratings (POMS). Repeated measures ANCOVAS were conducted for mean SBP, DBP and HR levels at baseline and during the task to determine reactivity to the tasks. NK cell activity (percent lysis) was calculated for each E:T ratio and differences between groups and changes from baseline within groups were analyzed using repeated measures MANCOVAs. Finally, hierarchical multiple regression analyses were conducted to examine the relationships of reactivity and self-reported distress with percent lysis. Repeated measures MANCOVAs were also conducted on lymphocyte proliferation to ConA and PWM data.

One-way analyses of variance comparing background characteristics across groups indicated that there were no differences in recent life events, daily hassles, symptom reporting or depression. Similar analyses indicated that there were no baseline differences in SBP, DBP, HR or immune measures. Analysis of the manipulation checks indicated that following the stressor period, both experimental groups reported higher levels of pain $F(2,27)=7.67$ $p<.01$, and distress $F(2,28)=14.95$ $p<.01$ than did control subjects, and the MA group

reported more depression on the POMS than did controls $F(2,26)=4.80$ $p<.01$. In addition, repeated measures ANCOVA of cardiovascular data yielded differences among groups for SBP ($F(2,28)=9.83$ $p<.01$), DBP ($F(2,28)=10.12$ $p<.01$) and HR ($F(2,28)=4.43$ $p<.02$). Post hoc analyses indicated that the MA group exhibited significantly higher SBP, DBP and HR during the task (p's<.05) than did the control group. SBP remained increased in the MA group at 5 minutes after the task ($F(2,29)=4.31$ $p<.03$). The CP group showed smaller, nonsignificant changes (see table 1).

Insert Table 1 about here

A repeated measures MANCOVA on percent NK lysis change from pre-stress levels, covarying for baseline, was performed for the 4 E:T ratios across the five blood draws by group. A nonsignificant group x time x dilution effect indicated a similar pattern across dilutions so the data were collapsed across dilutions. A significant group by time effect, $F(8,44)=2.8$, $p<.02$, suggested that the stressors affected NK lysis (see figure 1), and post hoc analyses indicated that the MA group showed a significant increase in NK activity at 2 min post-stressor initiation (PSI), 5 min PSI and 5 min after termination of the stressor (11 min PSI)(ps<.05). NK activity returned to baseline at 15 min post-termination. The CP group showed a marginal increase in NK activity 2 min after initiation of the stressor ($p<.07$) with an immediate return to baseline (see figure 1). Identical analyses were performed on raw levels of NK activity, and similar results were

obtained.

Insert Figure 1 about here

A hierarchical multiple regression analysis was conducted to examine the relationship between physiological arousal (as measured by SBP, DBP and HR) and peak NK cell cytotoxicity 2 minutes after stressor initiation. Baseline and peak NK cell cytotoxicity (at 2 minutes into the stressor) levels were averaged across the 4 E:T ratios, and after removing variance associated with baseline cytotoxicity and group, HR change was a significant predictor of peak cytotoxicity, accounting for 13% of the variance in NK cytotoxicity ($p<.01$). Peak NK activity and increased NK activity at 5 minutes into the stressor were also predicted by change in DBP from baseline to 2 min PSI, accounting for an additional 6.5% and 9.6% of the variance, respectively (p's$<.05$). These analyses indicated that higher DBP and HR change were positively correlated with NK cell cytotoxicity.

Due to considerable variance and nonnormal distribution of lymphocyte proliferation data, a logarithmic transformation was conducted on the data. Analyses were performed on change scores from baseline for each mitogen, and proliferation to Con A and PWM was analyzed separately. A repeated measures MANCOVA crossing group and mitogen concentration and covarying for baseline yielded a significant group x dilution x time effect, $F(16,40)=2.00$, $p<.04$. The MA group showed decreased proliferation to each Con A concentration during the stressor (2 min PSI & 5 min PSI), while a decrease in proliferation was

observed at 2 min PSI with a more rapid return to baseline for the CP group. However, post hoc analyses were nonsignificant primarily due to unexpected fluctuations in the control group data.

An identical repeated measures MANCOVA was conducted on the three PWM dilutions and yielded a significant group x time effect, $F(8,46)=2.11$, $p=.05$, without a significant group x time x dilution effect (see figure 2). Overall, MA subjects exhibited larger decreases in proliferation than did others during stressor exposure, returning to baseline by the end of the study. Subjects in the CP group exhibited smaller decreases in proliferation that also returned to baseline.

Insert Figure 2 about here

Discussion

This study addressed two main objectives: the examination of temporal characteristics of stressor-induced immune changes, and the replication of earlier findings regarding proliferation differences across stressor type. Immune reactivity to two common laboratory stressors (MA and CP) was studied during and shortly after stressor exposure, and NK cell activity was found to increase immediately in both groups and to remain increased in the MA group until 15 minutes after the stressor. Lymphocyte proliferation, although considerably variable, indicated that both MA and CP subjects exhibited decreases in proliferation to PWM with greater decreases in the MA group.

Many investigators have reported varying NK activity in response to acute

laboratory stressors. While some of this variance is likely due to the use of different stressors, we hypothesized that NK activity might follow a biphasic pattern similar to that associated with opponent processes. That is, changes associated with acute stressor exposure cause increases in NK function, but counteracting changes quickly push NK cell activity back toward or below baseline or resting levels. Depending on the timing of measures during and after stressor exposure, increases or decreases in activity could be observed. In the present study, subjects exposed to mental arithmetic showed an immediate increase in NK activity during the stressor that returned and fell below baseline values 15 min after termination of the stressor. The CP group showed a marginal increase in NK activity during the stressor and a rapid return to below baseline levels.

The decrease in activity at the end of the experiment was not significant, and this may be because we did not continue measurement long enough. Studies reporting a decrease in NK activity have observed this decrease at more distal timepoints than the ones we measured (Sieber et al., 1992; Cohen et al., under review). Had we measured NK activity at 30-60 min post-stressor termination we might have observed the anticipated decrease in activity. For instance, Cohen et al. (under review) exposed subjects to a mild 30-minute stressor and took immune measures at 10 and 20 minutes into the stressor, at the end of the stressor, and 40 minutes after stressor exposure. They found no effects of the stressor 10-30 minutes after initiation of the stressor, presumably because increases had already occurred and pressures towards equilibrium had already intervened. However,

they found significantly decreased NK activity at the 40 minute post-task measurement among subjects who were exposed to the stressor. Had they taken immune measures immediately after initiation of the stressor period, they may have observed an initial increase, as we did. These results, combined with those of the present study, suggest a biphasic pattern of NK activity in response to an acute stressor.

The data for lymphocyte proliferation were disappointing. We were unable to show differential blastogenic response patterns during or after the two stressors. The proliferation data suggest that the CP group behaved as if it was exposed to a milder degree of stress than the MA group rather than to a different type of stressor. This pattern held for all of our measurements. The CP group showed smaller but similar changes in NK activity during the tasks, and though subjects in the CP and MA groups rated their tasks as equally stressful, cardiovascular responses exhibited in the MA group were significantly greater during the stressor. A possible explanation for these findings in that we used a short 6-minnute cold pressor task whereas previous studies (Zakowski, 1993) have found changes in proliferation after 20 minutes of an unpredictable cold pressor task.

Consistent with previous studies (Cohen et al., under review; Manuck et al., 1991; Zakowski et al., 1992) we found a relationship between cardiovascular measures during the task and immune response. However, this relationship was positive, with greater increases in HR and DBP from baseline to 2 min PSI predicting greater increases in NK activity during the stressor. Cohen et al. (under

review) found a relationship between heightened cardiovascular reactivity and a distal decrease in NK activity. Although these results seem contradictory, they are consistent with studies that have shown that *in vivo* administration of epinephrine to humans resulted in an immediate increase in NK activity followed by a subsequent decrease (Tonnesen, Christensen & Brinklov, 1987; Kappel et al., 1991).

This study provided a picture of immune activity in response to two common acute laboratory stressors. Unfortunately, the lymphocyte proliferation data was not as strong as we had wished. We were unable to replicate Zakowski et al.'s (in press) findings of differential effects of the two stressor types of proliferation. This may be because the cold pressor task did not elicit as large a stress response as had been previously found in our laboratory, perhaps due to the shorter task duration. Zakowski et al. (1993) found significantly decreased proliferation to ConA after 20 minutes of an unpredictable cold pressor task and not at 10 minutes into the task indicating that perhaps our CP task period was not long enough to differentially affect immune activity. However, the significant group x time effect for proliferation to PWM indicated that the task period did elicit a change in proliferation, and further analysis showed that proliferation was decreased during the stressors in both experimental groups.

The pattern of NK response we observed is typical of an opponent process, but future studies should measure more distal timepoints post-stressor to determine if a significant rebound decrease in activity occurs. If so, this would

explain the variation in NK activity results reported in the literature. In addition, if there is a delayed decrease in activity following stressor exposure, the extent and duration of this decreased period should be examined. Future studies examining NK cell activity should take into account the pattern of NK response to stressors and plan blood draw times accordingly.

References

Bachen, E., Manuck, S., Marsland, A., Cohen, S., Malkoff, S., Muldoon, M. & Rabin, B. (1992). Lymphocyte subset and cellular immune responses to a brief experimental stressor. Psychosomatic Medicine, 54, 673-679.

Beck, A., Ward, C., Mendelson, M., Mock, J. & Erbaugh, J. (1961). An inventory for measuring depression. Archives of General Psychiatry, 4, 53-61.

Ben-Eliyahu, S., Yirmiya, R., Liebeskind, J., Taylor, A., & Gale, R. (1991). Stress increases metastatic spread of a mammary tumor in rats: evidence for mediation by the immune system. Brain, Behavior, and Immunity, 5, 193-205.

Brosschot, J., Benschop, R., Godaert, G., DeSmet, M., Olff, M., Heijnen, C. & Ballieux, R. (1992). Effects of experimental psychological stress on distribution and function of peripheral blood cells. Psychosomatic Medicine, 54, 394-406.

Cohen, L., Delahanty, D.L., Schmitz, J.B., Jenkins, F.J. & Baum, A. (under review). The effects of mild stress on natural killer cell activity in healthy men.

Cohen, S., Tyrrell, D. & Smith, A. (1991). Psychological stress and susceptibility to the common cold. The New England Journal of Medicine, 325, 606-612.

Derogatis, L. R. (1977). SCL-90-R: Administration Scoring and Procedures Manual 1. Baltimore: Clinical Psychometrics Research.

Dorian, B. & Garfinkel, P. (1987). Stress, immunity and illness- a review. Psychological Medicine, 17, 393-407.

Eichman, W. (1978). In Buros, O. (Ed.) The Eighth Mental Measurements Yearbook (p. 1016). New Jersey: The Gryphon Press.

Herberman, R. & Ortaldo, J. (1981). Natural killer cells: their role in defenses against disease. Science, 214, 24-29.

Herbert, H. T. & Cohen, S. (1993). Stress and immunity in humans: a meta-analytic review. Psychosomatic Medicine, 55, 364-379.

Irwin, M., Daniels, M., Smith, T., Bloom, E. & Weiner, H. (1987). Impaired natural killer cell activity during bereavement. Brain, Behavior, and Immunity, 1, 98-104.

Jemmott, J. & Locke, S. (1984). Psychosocial factors, immunologic mediation, and human susceptibility to infectious diseases: how much do we know? Psychological Bulletin, 95, 78-108.

Kappel, M., Tvede, N., Galbo, H., Haahr, P.M., Kjaer, M., Linstow, M., Klarlund, K. & Pedersen, B.K. (1991). Evidence that the effect of physical exercise on NK cell activity is mediated by epinephrine. Journal of Applied Physiology, 70, 2530-2534.

Kasl, S., Evans, A. & Niederman, J. (1979). Psychosocial risk factors in the development of infectious mononucleosis. Psychosomatic Medicine, 41, 445-466.

Kiecolt-Glaser, J. K., Cacioppo, J. T., Malarkey, W.B. & Glaser, R. (1992). Acute psychological stressors and short-term immune changes: what, why, for whom, and to what extent? Psychosomatic Medicine, 54, 680-685.

Kiecolt-Glaser, J.K., Garner, W., Speicher, C., Penn, G.H., Holliday, J. & Glaser, R. (1984). Psychosocial modifiers of immunocompetence in medical students. Psychosomatic Medicine, 46(1), 7-14.

Knapp, P.H., Levy, E., Giorgi, R.G., Black, P.H., Fox, B.H. & Heeren, T.C. (1992). Short-term immunological effects of induced emotion. Psychosomatic Medicine, 54, 133-148.

Landmann, R., Muller, F., Perini, C., Wesp, M., Erne, P. & Buhler, F. (1984). Changes of immunoregulatory cells induced by psychological and physical stress: relationship to plasma catecholamines. Clinical and Experimental Immunology, 58, 127-135.

McNair, D.M., Lorr, M. & Proppleman, L.F. (1971). Profile of Mood States. San Diego, CA: Educational and Industrial Service.

Naliboff, B., Benton, D., Solomon, G., Morley, J., Fahey, J., Bloom, E., Makinodan, T. & Gilmore, S. (1991). Immunological changes in young and old adults during brief laboratory stress. Psychosomatic Medicine, 53, 121-132.

O'Leary, A. (1990). Stress, emotion, and human immune function. Psychological Bulletin, 108, 363-382.

Riley, V. (1981). Psychoneuroendocrine influences on immunocompetence and neoplasia. Science, 212, 1100-1109.

Schedlowski, M., Jacobs, R., Stratmann, G., Richter, S., Hadicke, A., Tewes, U., Wagner, T.O.F. & Schmidt, R.E. (1993). Changes of natural killer cells during acute psychological stress. Journal of Clinical Immunology, 13, 119-126.

Schneiderman, N. & McCabe, P.M. (1989). Psychophysiologic strategies in laboratory research. In N. Schneiderman, S. M. Weiss, and P. G. Kaufman (eds.) Handbook of Research Methods in Cardiovascular Behavioral Medicine. (pp. 349-364). New York: Plenum Press.

Sieber, W., Rodin, J., Larson, L., Ortega, S., Cummings, N., Levy, S., Whiteside, T. & Herberman, R. (1992). Modulation of human natural killer cell activity by exposure to uncontrollable stress. Brain, Behavior, and Immunity, 6, 141-156.

Shimokawara, I., Imamura, M., Yamanaka, N., Ishii, Y. & Kikuchi, K. (1982). Identification of lymphocyte subpopulations in human breast cancer tissue and its significance: an immunoperoxidase study with anti-human T- and B- cell sera. Cancer, 49, 1456-1464.

Sklar, L. S. & Anisman, H. (1981). Stress and cancer. Psychological Bulletin, 89, 369-406.

Solomon, R. & Corbit, J. (1974). An opponent-process theory of motivation: 1. temporal dynamics of affect. Psychological Review, 81, 119-145.

Steinhauer, E., Doyle, A., Reed, J. & Kadish, A. (1982). Defective natural cytotoxicity in patients with cancer: normal number of effector cells but decreased recycling capacity in patients with advanced disease. The Journal of Immunology, 129, 2255-2259.

Tonnesen, E., Christensen, N.J. & Brinklov, M.M. (1987). Natural killer cell activity during cortisol and adrenaline infusion in healthy volunteers. European Journal of Clinical Investigation, 17, 497-503.

Weisse, C., Pato, C., McAllister, C., Littman, R., Breier, A., Paul, S. & Baum, A. (1990). Differential effects of controllable and uncontrollable acute stress on lymphocyte proliferation and leukocyte percentages in humans. Brain, Behavior, and Immunity, 4, 339-351.

Zakowski, S.G. (1993). The effects of stressor predictability on lymphocyte proliferation in humans. Dissertation Abstracts. University of Michigan, Ann Arbor, Michigan.

Zakowski, S.G., Cohen, L., Hall, M.H., Wollman, K. & Baum, A. (in press). Differential effects of passive and active laboratory stressors on immunity. International Journal of Behavioral Medicine.

Zakowski, S.G., McAllister, C.G., Deal, M. & Baum, A. (1992). Stress, reactivity, and immune function in healthy men. Health Psychology, 11, 223-232.

Figure 2. Change in proliferation from baseline (log transformation) for three concentrations of PWM.

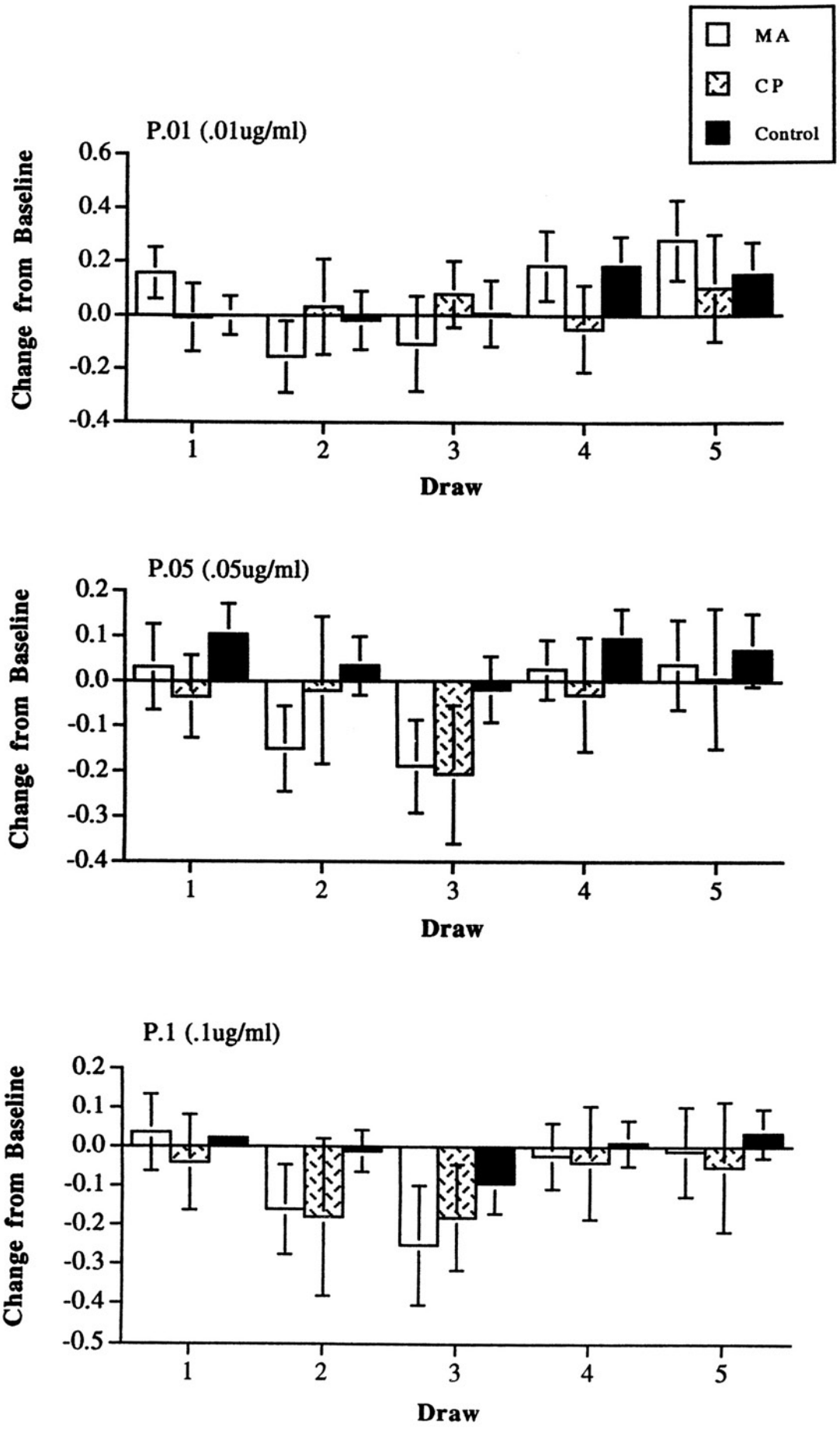
MA
CP
Control
P.01 (.01ug/ml)
Change from Baseline
0.6
0.4
0.2
0.0
-0.2
-0.4
1
2
3
4
5
Draw
P.05 (.05ug/ml)
Change from Baseline
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
1
2
3
4
5
Draw
P.1 (.1ug/ml)
Change from Baseline
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
1
2
3
4
5
Draw

Figure 1. Mean NK activity levels (% lysis) for all groups.

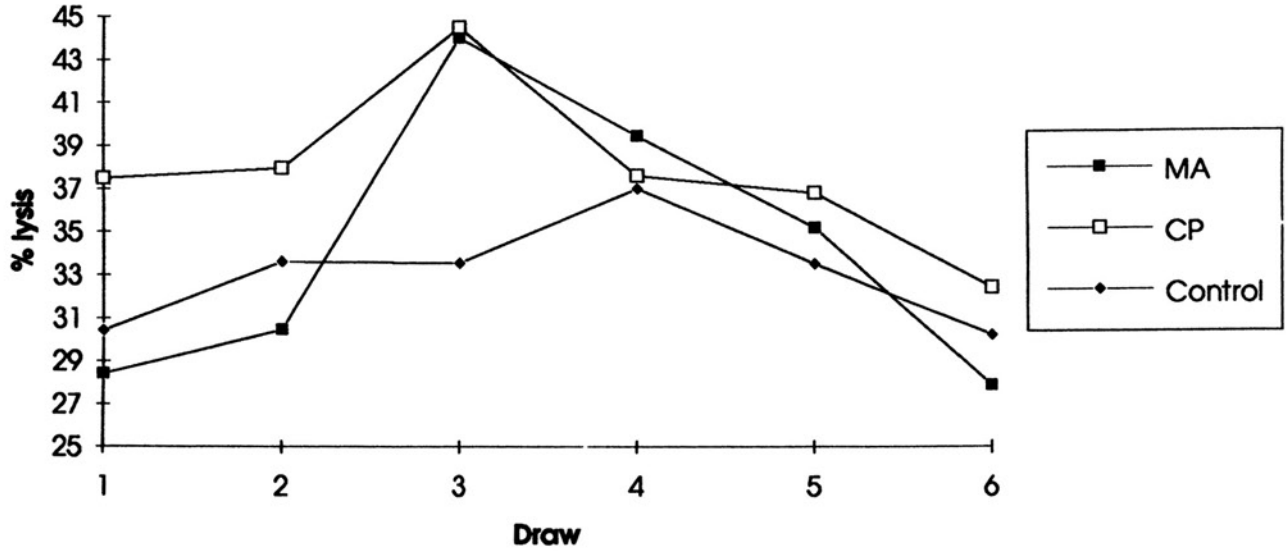

Table One: Means and standard deviations for SBP and DBP (in mm Hg) and HR (in beats per minute) at baseline and during the task.

	SBP		DBP		HR	
	BASELINE	TASK	BASELINE	TASK	BASELINE	TASK
MA GROUP	124.72 ± 10.85	142.61 ± 9.67	76.44 ± 5.80	86.27 ± 6.41	65.26 ± 7.96	82.22 ± 19.78
CP GROUP	126.22 ± 11.25	131.80 ± 13.12	74.38 ± 7.32	78.78 ± 8.10	61.68 ± 6.85	65.38 ± 14.60
CONTROLS	118.60 ± 9.05	118.68 ± 9.29	70.71 ± 7.36	70.39 ± 7.88	67.76 ± 5.98	68.26 ± 8.48